Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

VOLUME XXX NUMBER 2

BOTANICAL GAZETTE

AUGUST, 1900

THE DEVELOPMENT AND FUNCTION OF THE CELL PLATE IN HIGHER PLANTS.

H. G. TIMBERLAKE.

(WITH PLATES VIII AND IX)

Historical.

ASIDE from the work of Strasburger there have been very few investigations reported that have treated fully the subject of the cell plate and its history in the vegetable cell. So far as I know, the term cell plate was first used in its present sense by Strasburger in the first edition of *Zellbildung und Zelltheilung*. It was here that he made the statement that the beginning of the cell plate is to be found in swellings of the connecting spindle fibers. The subject was more fully discussed in the third edition of the above work, and I shall refer to that in greater detail below.

In 1878 Treub published his classic researches on the rôle of the nucleus in cell division,[1] in which he describes the process of cell plate and cell wall formation in the living cells of the proembryo of *Orchis latifolia* and the ovules of *Epipactis palustris*. By keeping the tissues in a 1.25 per cent. solution of KNO_3 he was able to make an extended study of the above processes. The first indication of the formation of a cell plate is a collection of granules across the equatorial region of the spindle, which move into place from various directions in the cytoplasm. They have, therefore, no connection with the spindle fibers. After reaching the equator the granules fuse into a continuous layer across the central spindle. The growth of the cell plate occurs by the addition of new material either all around, if the spindle is in the middle part of the cell, or on the free sides, if the spindle is near one side of the cell so that the cell plate has reached the mother cell wall on that side where it is first formed, *i. e.*, the whole nuclear figure moves across the cell, building the cell plate as it goes. By plasmolyzing the cell by the addition of more KNO_3 Treub was able to demonstrate that the new cell wall is laid down between the split halves of the cell plate.

---

[1] Quelques recherches sur le rôle du noyau dans la division des cellules végétales. Amsterdam, 1878.

In the third edition of *Zellbildung und Zelltheilung* Strasburger gave the most comprehensive account of the cell plate that has ever appeared. One of the important facts that he describes in this work is the apparent increase in the number of connecting fibers prior to the formation of the cell plate (*op. cit.*, p. 341). There are a few spindle fibers extending through the cell plasma between the receding groups of daughter chromosomes. These fibers can be distinguished from the surrounding cell plasma by their filar form. Their number is increased by the addition of new fibers differentiated out of the cytoplasm. The new fibers are identical, in so far as their staining qualities and form are concerned, with the original fibers. This apparent identity is evidence for the cytoplasmic origin of spindle fibers. The hypothesis is strengthened by the process of division of those pollen mother cells which divide simultaneously, where new connecting fibers are formed from the cytoplasm after the two daughter nuclei of the first division have again divided, and the original connecting fibers, with the cell plate formed in them, have disappeared. The permanent cell plate is then formed in these new fibers (*op. cit.*, p. 345). In the spore mother cells of Anthoceros and the macrospore mother cells of Isoetes, the spindle fibers in which the cell plates are formed connect the four daughter plasma masses or chromatophores, instead of the nuclei. Davis[2] has recently reinvestigated the division of the spore mother cell of Anthoceros and has found that the cell plate is formed upon cytoplasmic strands (not spindle fibers) uniting the four chromatophores. A portion of this cell plate becomes converted into a cell wall.

---

[2] The spore mother cell of Anthoceros. BOT. GAZ. **28**: 89. 1899.

In endosperm formation there are a great number of nuclear divisions that are not followed directly by divisions of the cell. The spindle in each case disappears after each nucleus divides.[3] When the above process has continued until there are a great many nuclei lying free in the protoplasm, new fibers are formed connecting each nucleus with all of its immediate neighbors. In these fibers the cell plates are built in the usual manner. In a later work[4] these new fibers were described as growing out of a hyaline plasma mass around each nucleus.

On the nature of the cell plate elements, *i. e.*, the granules which form the cell plate, we have an interesting statement.[5] The cell plate is formed of small granules whose chemical nature is hard to determine. That they may be starch is indicated by the fact that they take in some cases a blue stain when treated with iodine. In most cases, however, they are not thus stained. They may be a substance between starch and cellulose. This hypothesis is rendered more probable by the fact that the cell plate elements are apparently used directly to form a cellulose wall instead of being converted into a protoplasmic layer which splits and excretes a cellulose layer between the halves.

In the pollen mother cells of the Cycadaceæ, Juranyi[6] described a process of cell wall formation by the conjunction of a cellulose ring, growing in from the mother cell wall and a new wall formed in the connecting spindle.

In 1882 Strasburger modified his previous view as to the origin and chemical nature of the cell plate elements.[7] In this paper he holds that with the aid of suitable stains it can be shown that the granules of the cell plate react as albumen. As to the origin of these granules it was observed that they are not added from the surrounding protoplasm but are within the fibers themselves. The fibers appear as if made up of small granules. These granules are really contained in the fibers as if in tubes, and they collect at the equator, forming apparent equatorial swellings of the fibers, *i. e.*, the cell plate elements. The cell plate elements, after growing by the addition of new granules, finally fuse into a continuous plate.

---

[3] STRASBURGER, *op. cit.*, p. 345.

[4] Ueber Kern- und Zelltheilung im Pflanzenreich. Hist. Beitr. 1. 1888.

[5] Zellbildung und Zelltheilung, 3. Aufl., 342.

[6] Mittheilungen über Structur u. Bildung des Zellkernes. Ref. in Botan. Centralb. **12**:213. 1882.

[7] Ueber den Bau u. d. Wachsthum der Zellhäute 172. Jena, 1882.

In 1887 Went[8] showed that connecting fibers and other spindle fibers are identical, and that the former apparently increase in number before the cell plate appears. He was not able, however, to account for the origin of the new fibers. Prior to the formation of the cell plate, Went observed a stained substance between the fibers of the connecting spindle next to the daughter nuclei, leaving thus a clear zone in the equator of the spindle. This stained substance was thought to be nucleolar matter dissolved in the nuclear sap. In the subsequent stages these two darker zones move to the equator where the cell plate is built. Its origin Went did not see, but he showed that its growth is peripheral, as had been well established by previous observers. Went, however, observed for the first time that the connecting fibers disappear in the center of the spindle during the growth of the cell plate, so that the peripheral fibers form a ring connected with the growing region of the plate.

Opposed to the view of Strasburger as to the cytoplasmic origin of the cell plate by fusion of swellings of the connecting fibers, was that of Zacharias,[9] who contended that the spindle is of nuclear and the cell plate of cytoplasmic origin, thus following Treub's theory of the origin of the cell plate from free granules. The cell plate is formed by the entrance of substance from the surrounding protoplasm into the equatorial region of the central spindle, which is a portion of the dividing nucleus

---

[8] Beobachtungen über Kern u. Zelltheilung. Ber. d. deutschen botan. Ges. 247. 1887.

[9] Ueber Kern u. Zelltheilung. Botanische Zeitung **46** : 56. 1888.

(*Mutterkernrest*) having a homogeneous structure and hyaline appearance. In a later contribution he ascribed the filar structure of the central spindle to the effect of reagents, especially absolute alcohol.

In connection with his researches on fertilization Guignard[10] observed that the number of connecting fibers is equal to the number of chromosomes, and that these fibers are formed by the fusion of smaller primary fibers.

Later Strasburger[11] confirmed the views of Went as to the staining properties of the nuclear sap and the relation of the substance in it to cell plate formation. He also described the formation of a temporary plasma membrane about the connecting spindle, and suggested that the change in the form of the nuclear figure, *i. e.*, the bulging out of the connecting fibers, is due to the osmotic action of the nuclear sap held within this membrane.

In 1893 Wildeman[12] described in the rhizoids of mosses a process of division wall formation in which the spindle is first arranged so that its long axis is parallel to the long axis of the cell. Previous to the formation of the cell plate, it is changed to an oblique direction, but the young cell wall is so curved as to become attached perpendicularly to the mother cell wall in accordance with Sachs's law of "orthogonal trajectories."

In 1895 Strasburger[13] claimed new evidence for the identity in character of connecting fibers and other spindle fibers in the fact that their staining qualities were the same. In preparations stained with the triple stain of Flemming he observed that, while the cell plate is being formed, the connecting fibers go through all gradations from violet (the color of spindle fibers) to brown (the cytoplasmic color), thus confirming his earlier opinion as to the cytoplasmic nature of spindle fibers. From the fact that shortly before the cell plate is formed "extra-nuclear nucleoli" appear in large numbers in the equatorial region of the spindle, and that by the time the cell plate is complete these have disappeared, he argues that the nucleolus takes part in building the cell plate.

---

[10] Nouvelles études sur la fecondation. Ann. des Sci. Nat. Bot. VII. **14** : 163.

[11] Histologische Beiträge 1 : 162 *et seq.*

[12] Études sur l'attache des cloisons cellulaires. Mémoires couronnés et mémories d. sav. etr. p. p. l'Acad. Roy. d. Sci. de Belgique **53** : 19. 1893.

[13] Karyokinetische Probleme. Jahrb. f. wiss. Bot. **28** : 193. 1895.

In the pollen mother cells of *Lilium Martagon*, Farmer[14] found that the protoplasm of the connecting spindle on each side of the newly formed cell plate is relatively transparent.

The series of contributions which appeared from the Bonn laboratory in 1897 presented several important facts in regard to the formation of the cell plate. Mottier[15] found in the pollen mother cells of the lily that the cell plate is split into plasma membranes before the cell wall is laid down. In cells plasmolyzed in fixing, a complete plasma membrane was seen around each daughter cell, while no cellulose wall could be observed (*op. cit.*, p. 192). In the pollen mother cells of *Hemerocallis fulva*, Juel[16] found that after the first nuclear division the cell plate did not reach the mother wall but remained free in the cytoplasm until the second nuclear division occurred and new cell plates were formed at right angles to it. Then some of the fibers radiating from the daughter nuclei become attached to the old plate, and all the cell plates now continue their respective growths toward the mother cell wall. Debski[17] studied the division of the segment cells of Chara and observed that the long axis of the spindle is parallel to the shorter axis of the cell and further that the cell plate is not formed across the equator of the spindle but nearer one nucleus. In the formation of the ascospores in the asci of Erysiphe and Peziza, Harper[18] found that the plasma membrane around each ascospore was formed by the growth and lateral fusion of the polar radiations, a process which is essentially similar to the formation of the cell plate in higher plants.

---

[14] Kerntheilung in Lilium Antheren, besonders in Bezug auf die Centrosomenfrage. Flora **83**: 167. 1895.

[15] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen u. Monokotylen. Jahrb. f. wiss. Bot. **30**: 169. 1897.

[16] Die Kerntheilungen in den Pollenmutterzellen von *Hemerocallis fulva*, und die bei denselben auftretenden Unregelmässigkeiten. Jahrb. f. wiss. Bot. **30**: 205. 1897.

[17] Beobachtungen über Kerntheilung bie *Chara fragilis*. Jahrb. f. wiss. Bot. **30**: 227. 1897.

[18] Kerntheilung und freie Zellbildung im Ascus. Jahrb. f. wiss. Bot. **30**: 249. 1897.

In the division by which the "beaks" are cut off from the gametes in Basidiobolus, a typical cell plate is formed, according to Fairchild,[19] from two rows of granules, whose origin was not discovered, in the equatorial region of the spindle. In some of the Sphacelariaceæ Swingle[20] confirmed the previous observations of Strasburger, that the cell plate is not formed in a connecting spindle, but arises in the cytoplasmic network. Swingle suggested, however, that its formation may be under the control of the nuclei by means of the kinoplasmic fibers which radiate from the poles, although there are not enough of such fibers actually to form it. An important point in this connection is the fact that if the daughter nuclei are of unequal size the cell plate is formed nearer the smaller nucleus, as Debski described for Chara. In the oogonium of Fucus, Strasburger[21] found a cell plate built of granules which he considers extra nuclear nucleoli arranged in the cytoplasm. The granules divide so as to form two layers out of which are formed continuous membranes. Discussing the subject more generally, Strasburger[22] places great emphasis upon the kinoplasmic nature of the cell plate. He points out the fact that the formation of a cell plate by the fusion of swellings of the connecting fibers is analogous to the formation of the plasma membrane around the ascospores as described by Harper. In the case of the ascospores there is no doubt of a transformation of the substance of the fibers (polar radiations) into a plasma membrane. In the case of the cell plate, while it is equally evident that there is a transformation of fiber substance into a lamella from which is derived the plasma membrane of the daughter cells, Strasburger was not then certain whether the cell plate forms simply the plasma membranes, or whether a part of it may be changed into a division wall (*op. cit.*, p. 38). In a subsequent contribution,[23] however, he made the statement that the cell plate forms a plasma membrane (*Hautschicht*), that it splits to form a plasma membrane for each daughter cell, and that the substance of the cellulose wall is excreted by these daughter plasma membranes and laid down as a wall between them.

---

[19] Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum* Eidam. Jahrb. f. wiss. Bot. **30**: 285. 1897.

[20] Zur Kenntniss der Kern- und Zelltheilung bei den Sphacelariaceen. Jahrb. f. wiss. Bot. **30**: 297. 1897.

[21] Kerntheilung und Befruchtung bei Fucus. Jahrb. f. wiss. Bot. **30**: 351. 1897.

[22] Ueber Cytoplasmastructuren im Kern- u. Zelltheilung. Jahrb. f. wiss. Bot. **30**: 375. 1897.

Hof[24] has recently described in the division of vegetative cells what I take to be the same appearance as that described by Went and Strasburger, viz., the deeply stained portions of the spindle moving toward the equator and there taking part in the formation of the cell plate. He, however, describes the substance of these zones as granular rather than as material in solution in the nuclear sap. After the cell plate is complete the connecting fibers are entirely drawn in (*eingezogen*) and their place taken by alveolar cytoplasm. Just what Hof means by the drawing in of the connecting fibers is a question left unsettled in his description. They may be drawn into the nucleus or into the cell plate. His figures do not help at all in understanding his meaning in this instance.

Nemec[25] has recently described the same phenomena as to the formation of the cell plate in the growing root tips of *Allium Cepa*. He adds further that new connecting fibers make their appearance after these zones have reached the equator. The fact that these new fibers often seem to end free between the original fibers, Nemec considers as evidence for their origin from the stained substance now at the equator.

The observations of Strasburger as to the method of cell plate formation in the oogonium of Fucus were not wholly confirmed by Farmer and Williams.[26] These investigators found that there is an accumulation of material, which they think is some form of a carbohydrate, in the neutral zone between the daughter nuclei of the future oospheres. There is, however, no trace of a preliminary cell plate. While spindle fibers were seen reaching from the poles to the equatorial region of the cell, no evidence was found that they take part formatively in the building of the division wall.

---

[23] Zellhäute. Jahrb. f. wiss. Bot. **31** : 514. 1898.

[24] Histologische Studien am Vegetationspunkten. Bot. Centralb. **76** : 221. 1898.

[25] Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*. Jahrb. f. wiss. Bot. **32** : 313. 1899.

In an early paper Strasburger had pointed out the difference between the method of division of the cell body of animal cells and that of the higher plants, by stating that in the former the connecting spindle fibers take no part in the division of the cell body, but that the division is accomplished by the constriction of the plasma membrane; whereas in the latter these fibers are increased in number and take part in the division of the cell by forming a cell plate. This view Carnoy[27] attempted to overthrow in his work on the testicular cells of some arthropods, in which he claimed to have found a cell plate formed of two parts, a cytoplasmic part formed by thickenings appearing on the junctures of the cytoplasmic network, and a spindle part formed by swellings on the connecting spindle fibers. I have not been able to find that this work has been confirmed by subsequent investigators. The spindle portion (*plaque fusoriale*) of Carnoy is undoubtedly the midbody (*Zwischenkörper*) subsequently described by Flemming[28] and others as arising from granules in the equatorial region of the spindle, but not always as swellings of the spindle fibers. Notwithstanding the fact that it takes no part in the division of the cell body, Flemming was inclined to consider it the homologue of the cell plate in the higher plants. The midbody has received a great deal of attention from the zoologists, and various views have been held as to its significance and fate. The literature on the subject has been recently succinctly reviewed by Ballowitz,[29] so that I need not go over it here. Ballowitz finds that the midbody is formed by the fusion of swellings of the central spindle fibers. His observation differs from Flemming's in that the latter thought the granules were sometimes between the fibers. Ballowitz and Flemming agree that the midbody takes no part in the division of the cell, but may be seen between the two daughter cells after division is complete. In a very interesting paper by Kostanecki,[30] upon the relation of polar radiations to the division of the cell body, the view is developed that the division is effected by a cell plate built in the cytoplasm by means of two systems of polar radiations which connect the granular cell boundary with the centrosomes. During the metaphases the longer fibers from each centrosome, which cross each other in the equatorial region of the cell, contract and so change their points of attachments to the plasma membrane until they reach the equator. They now draw in toward the central spindle, pulling in with them a substance similar to that of the cell boundary, thus forming what the author calls an equatorial granular cell plate. The division of the cell depends primarily upon the differentiation of the cell plate into two layers under the influence of the opposite system of polar radiations. Kostanecki states further that he considers the cell boundary to be a material similar to that of the fibers. This hypothesis agrees very well with what we know of the formation of the plasma membrane in the higher plants and asci, as will be seen later. It is perhaps the first suggestion on the part of investigators of the animal cell that the two substances are the same.

---

[26] Contributions to our knowledge of the Fucaceæ. Philos. Trans. of the Roy. Soc. London B. **190**: 623.

[27] La cytodiérèse chez les arthopodes. La Cellule **1**: 375.

[28] Neue Beiträge zur Kenntniss der Zelle. Arch. für mikr. Anat. **38**: 690.

Descriptive.

The present investigation was undertaken to attempt to determine in detail the exact sequence of events during the division of the cell body, and to correlate, so far as possible, the facts thus brought out from the point of view of the physiology of cell reproduction.

---

[29] Zur Entstehung des Zwischenkörpers. Anat. Anz. **14**: 390.

[30] Ueber die Bedeutung der Polstrahlung während der Mitose u. ihr. Verhältniss zur Theilung der Zellleibes. Arch. f. mikr. Anat. **49**: 651.

MATERIAL.

My observations were made largely upon (1) vegetative cells found in the growing root tips of various phanerogamic plants, among which may be mentioned *Allium Cepa*, *Lilium longiflorum*, *Fritillaria imperialis*, *Hyacinthus orientalis*, *Vicia Faba*, *Phaseolus vulgaris*, *Pisum sativum;* and (2) pollen mother cells of *Larix Americana* and *Larix Europæa*, and dividing pollen grains of *Iris versicolor* and *Hemerocallis fulva*.

In the first group I found the liliaceous plants were much more favorable for observation, and of these, owing to the ease with which the material could be obtained, *Allium Cepa* was the one generally used. I have satisfied myself, however, that the phenomena I shall describe occur in all of the plants mentioned, and my conclusions are of course drawn from a study of all of these plants. In many particulars there is a notable difference between the phenomena that may be seen in such tissues as the root tip and such cells as the pollen mother cells of the larch. These differences I shall attempt to correlate to some extent with the nature of the cells.

METHODS.

As some of the phenomena herein recorded are at variance with those observed by previous investigators, I have thought it worth while to give a detailed account of the methods used, even though they are those already well known to cytologists. Various killing and fixing methods were employed. Flemming's chrom-osmo-acetic acid (both formulas); Hermann's platinum chlorid-chrom-acetic acid; Vom Rath's platinum chlorid-picro-osmo-acetic acid; Keiser's mercuric chlorid-acetic acid; 96 per cent. alcohol, and a mixture of mercuric chlorid, formalin, acetic and formic acids arranged by Professor D. C. Worcester, of the department of zoology of the University of Michigan, and soon to be published by him. Of these methods, the one best adapted to my purpose was the stronger solution of Flemming, since the kinoplasmic portions of the cell in division were nearly always well fixed in it. It proved in this respect to be much superior to the weaker solution for the tissues studied. The material was kept in the killing fluid 30–48 hours, washed in running water, hardened by carrying through the different grades of alcohol and imbedded in paraffin in the usual way. Some care was necessary to avoid plasmolysis in the early stages of hardening. I found that the objects could not be left in the lower grades of alcohol for a very long time; 30 minutes in 15 per cent., 45 minutes in 30 per cent., and one hour in 50 per cent. seemed to be about the maximum limits. Above 50 per cent. more latitude as to time could be allowed.

Vom Rath's and Hermann's solutions generally fixed the material well, but were not so useful as Flemming's solution on account of the blackening of the tissue and the consequent difficulty in getting a clear differentiation in staining. The method of Professor Worcester was found to be useful for comparative purposes. For these tissues it has no other special value. Alcohol (95 per cent.) was irregular in its effects, sometimes giving well-preserved material but more frequently causing plasmolysis. The other methods were found generally unsatisfactory.

The sections (generally 5μ thick) were cut on a microtome and stained on the slides. The method best adapted to use in connection with objects killed in fluids containing osmic acid was the safranin-gentian-violet-orange method of Flemming. After removal of the paraffin the slides were placed in the safranin solution 25–30 minutes, rinsed in water, and then put into the gentian-violet solution 20–25 minutes. They were again rinsed in water and passed rapidly through a *weak* orange solution (1 part saturated solution orange G in H_2O + 1 part H_2O), dehydrated, clarified quickly in clove oil, and mounted in balsam. For staining objects killed in the fluids containing mercuric chlorid, Zimmermann's fuchsin iodine green[31] and Heidenhain's haematoxylin, preceded by Bordeaux red as a ground stain, were found most serviceable.

---

[31] Morph. u. Phys. d. pfl. Zellkernes 6.

In order to show clearly certain zonal differentiations in the spindle as well as other general structures in the cells I have used photo-micrographs for many of the illustrations. I have considered this the more satisfactory, inasmuch as my preparations have showed some phenomena varying markedly from the descriptions of other observers.

OBSERVATIONS.

For purposes of description we may group the phenomena under three heads representing three stages in the development of the cell plate, viz., *preparatory; genetic; growing.*

1. *The preparatory stage.*

At the time when the chromosomes have collected at the equator to form the equatorial plate the spindle is of the form that Hof has recently termed "monaxial," *i.e.*, a spindle having a single axis but not necessarily ending in single definite points as poles (*figs. 1, 10, 23*). The poles of the spindle in both the larch and the onion are in most cases somewhat blunt. In the latter plant my observations agree entirely in this respect with those of Hof (*l. c.*) and Nemec (*l. c.*). In the case of the larch I have not confirmed Strasburger's[32] observation of a centrosphere. In my preparations the poles generally appear blunt as they do in the onion.

The fibers which appear in connection with this stage may be grouped into three systems, connecting fibers, mantle fibers, and radiating fibers. These systems correspond in general to the system of fibers similarly named by older investigators, especially among the zoologists, except that here, as will appear later, the distinction cannot be so sharply drawn between connecting fibers and radiating fibers. If we study the above mentioned systems of fibers more in detail, it will appear that while many of the connecting fibers are collected into thick strands or bundles, and others appear as single fibers, such an arrangement does not indicate a necessary difference of character. Very frequently a single fiber diverges from a bundle and unites with another bundle or continues as a single fiber to the pole. The arrangement into bundles seems to be produced partly, at least, by the fibers being crowded into the small spaces between the chromosomes. This explanation is rendered more probable by the fact that, as Strasburger has pointed out, the arrangement into bundles is much more evident at the equator than in the polar regions of the spindle (*op. cit.*, p. 183), *i. e.*, the fibers forming a single strand often seem to diverge beyond the equator. Some of my preparations show that this divergence reaches to the ends of the strand (*fig. 23*), so that such strands are most compact where they are adjacent to the chromosomes.

---

[32] Karyokinetische Probleme *fig. 25.*

The appearance of single fibers crossing obliquely from one bundle to another, together with the fact that such fibers often cross and recross each other, was taken by Belajeff as evidence that the fibers are drawn out portions of a protoplasmic net work. Guignard's observation that the number of connecting fibers is equal to the number of chromosomes may be explained by the hypothesis that the fibers are collected into larger strands in the spaces between the chromosomes. Guignard, as previously stated, described such fibers as secondary fibers formed by the lateral fusion of previously existing smaller primary fibers. He did not, however, distinguish any other than the connecting fibers. Strasburger later showed that there are other fibers extending from the poles to the chromosomes. These are the mantle fibers. They have been so clearly demonstrated recently by Osterhout, Mottier, Nemec, and others, that I need give no detailed description of them in this connection. Suffice to say, I find no apparent difference in structure between them and the connecting fibers.

The third system of fibers to which I have referred may be described briefly as including those which extend from the poles into the cytoplasm. They are much more abundant in the larch than in the onion; hence I shall first describe them as they appear in the former. They seem to have the same structure as the connecting or mantle fibers. Strasburger has figured them in the larch as radially arranged granules, while the connecting fibers are shown as continuous lines. In my preparations the granular trophoplasm through which the fibers extend often has a radial appearance where the fibers are very abundant, but it is always possible in good preparations to distinguish the single threads (*fig. 23*). It is only by means of their distribution and arrangement that I would distinguish the radiating fibers from the other systems. They may be said to be centered in a general way on each pole, and to radiate through the cytoplasm in all directions toward the cell boundary, but at this stage very few appear to reach the plasma membrane. Their arrangement around the poles is by no means regular. Some of them lie across the poles in such a way as to have both ends free in the cytoplasm. With these it is of course often difficult to determine whether a single fiber is seen, or whether the appearance is given by two fibers extending from the same point in opposite directions into the cytoplasm. In many cases, however, I have been able to satisfy myself that a single fiber extended through the pole and that its two ends lay free in the cytoplasm (*fig. 23*). This was most evident in cases where the fibers lay in such positions as to form appreciable angles with the axis of the spindle. Where the apparent radiating fibers are continuous or nearly so with the long axis of the spindle it is often difficult to determine whether they extend through the poles into the region of the spindle itself or whether they end at the poles. In some cases it appears as if such fibers are merely prolongations of the connecting or mantle fibers. I could not be certain on this point on account of the abundance of fibers in the polar regions of the spindle. Some, however, may be traced through the poles into the cytoplasm closely adjacent to the spindle. Still it can be readily observed that many of the radiating fibers which lie closely adjacent to the spindle do not extend beyond the poles These latter fibers bear an interesting relation to the connecting fibers. They often extend from the pole parallel to the connecting fibers, even uniting with the strands of the latter, nearly to the equator, where they curve outward and end blindly in the cytoplasm. Many of the fibers from each pole cross one another at the equator. These generally appear to be longer and more abundant than any of the other radiating fibers, but their identity in appearance with the connecting fibers makes it impossible in many cases to determine whether they extend to the poles or not.

From the above observations it seems to me to be improbable that there is any fundamental difference between the fibers of the three systems. While for convenience of discussion it is perhaps best to retain the classification heretofore used, it is worth while to understand clearly just what significance is to be attached to the different terms. By connecting fibers I mean those which lie entirely in the spindle and extend *across the equator*. Mantle fibers are those which lie entirely within the spindle and are attached to the chromosomes. The spindle is understood, then, to be made up of the mantle fibers and the connecting fibers. Radiating fibers are those which, connected in small part with the spindle, generally if not always at the poles have at least one end lying free in the cytoplasm. That this definition of radiating fibers may have to be slightly extended will appear later.

Whether there is any difference in origin of the various fibers I have not attempted to discover. The recent researches of Belajeff,[33] Osterhout, Mottier, and others, have indicated that all of the fibers have a common method of origin. Rosen,[34] however, believed from his study of spindle formation in the root tips of *Hyacinthus orientalis* that the connecting fibers are formed each by two fibers growing from opposite directions and fusing end to end at the equator. His observations have not been confirmed by later investigators.[35] So far as my observations go, in the onion radiating fibers seldom appear at this stage. Nemec, however, has described them as first appearing in the very early prophases and persisting through the metaphases. Their history, as Nemec describes it, is of much interest. The first indication of a spindle is an accumulation of hyaline substance around the nucleus, but in greater amount on the sides corresponding to the poles of the future spindle. The whole body thus forms an ellipsoid spindle fundament (*Anlage*). This soon shows a filar structure and in many cases these fibers extend outward from the sides and cross in the equatorial region of the cell. The radiating fibers thus appear prior to the other fibers, or it would perhaps be more accurate to say that they grow more rapidly, as rudiments of the other fibers appear at the same stage in the polar regions. It should be noted here that no radiating fibers are described for these early stages, except those extending toward the equator. Such fibers, however, reach to the plasma membrane and are attached to it. The attachment is shown by a small accumulation of violet-staining material at the point of juncture. While Nemec states that the above mentioned fibers persist throughout the process of nuclear division to the beginning of the anaphases, his figures of the equatorial plate stage do not show them, but in connection with the late metaphases they are again figured. It seems to me probable that the two sets of fibers are not identical. It is not unlikely that those first described changed their position and became connecting or mantle fibers, while the later radiating fibers are a new growth. Nemec himself states that he believes that some new radiating fibers are formed during the late metaphases. I have been able to see some radiating fibers in the onion in connection with the equatorial plate stage, but not in abundance nor of such length as Nemec described. They seldom reached beyond the equator. When they were present they showed the same relation to the other spindle fibers as has been described for the larch.

---

[33] Zur Kenntniss der Karyokinese be den Pflanzen. Flora **79**: 436. 1894.

[34] Kerne u. Kernkörperchen in meristematischen u. sporogenen Geweben. Cohn's Beiträge zur Biologie der Pflanzen **7**: 225.

[35] HOF, *loc. cit.*, and NEMEC, *loc. cit.*

The appearance of the cytoplasm in this stage requires a brief description. In the onion, it surrounds the spindle closely and may often be seen between the fibers in the polar region. It is generally slightly denser in the vicinity of the spindle than it is nearer the cell-membrane. In older cells there are frequent vacuoles in the outer portion. In the larch an interesting differentiation frequently occurs. About midway between the cell periphery and the spindle there is a thin layer of kinoplasmic material, looking as if it were made up of small portions of fibers in a tangled mass (*fig. 23*). A similar appearance was described by Mottier (*op. cit.*, p. 180) in an earlier stage of spindle formation in the pollen mother cell of the lily. He observed, however, that this layer forms a part of the spindle, *i.e.*, it is the same as the felted layer described by Strasburger and Belajeff. That it forms no part of the existing spindle in the larch, however, would be indicated by its late appearance after the spindle is formed. It may be that there is in the larch more kinoplasm formed than is needed for this stage in the development of the spindle, and that it becomes absorbed into the cell protoplasm or is used directly for the later stages in the growth of the fibers (p. 47.) Between this kinoplasmic layer and the spindle there often appears a thick layer of finely granular trophoplasm which stains readily with the orange. A similar layer in the pollen mother cells of Hermerocallis was described by Juel, but he he did not mention any strong affinity for the orange stain, such as I have observed in the larch. In some of my own preparations of Hermerocallis, I have observed the orange-stained layer very frequently.

Throughout the cytoplasm, and in some cases in the spindles also, there often appeared, in this as well as in later stages, large blue-stained granules. Their distribution was irregular. They were more apparent usually in the larch than in the onion, except during the metaphases, when they appeared very abundantly in the spindle of the latter. I shall discuss their appearance in connection with such stages in a subsequent paragraph.

In the early metaphases the connecting fibers appear of course much more plainly between the receding groups of daughter chromosomes. They can easily be traced through to each pole, thus being identified as the connecting fibers before mentioned and not as secondary structures formed simply between the groups of chromosomes. They show in the larch the compound structure before mentioned. In the onion, on the other hand, the portions between the receding groups of daughter chromosomes often appear as single coarse fibers. This appearance is probably due to the closer crowding of the small fibers by the chromosomes. It is possible that in some cases this crowding together has gone to such an extent as to result in an actual lateral fusion of the fibers, such as Guignard suggested. The above-described condition of connecting fibers is doubtless what led Nemec to conclude that during the metaphases there were new coarse fibers formed reaching merely between the daughter chromosomes. I have been able to determine that in most cases the fibers can be traced through to the poles, and that they often appear to separate into smaller fibers beyond the chromosomes in the way described by Strasburger for the larch. In the larch, in which the fibers show their compound character much more clearly, the true relation is still more distinctly seen (*fig. 2*). While I have not been able to confirm the observations of Nemec (*op. cit.*, p. 329) as to the presence of secondary fibers during the metaphases which are formed merely between the groups of daughter chromosomes, I have often observed that the above-mentioned blue-stained granules appear to be very numerous in the region of the spindle. They often appear in rows and sometimes seem to be sticking to the connecting fibers. But such a regular arrangement is not at all characteristic. They are as frequently scattered in the ground substance of the protoplasm (*fig. 25*). That they are formed from disintegrating secondary fibers seems impossible to believe when we consider the fact that they may be scattered throughout the whole cell as described above. As to the function or ultimate fate of these granules I could learn nothing definite. They seem to be distinct from the ordinary granules of the trophoplasm in staining qualities alone. The fact that they showed most plainly in the region of the spindle may simply mean that they are more readily distinguished by form from the spindle fibers, which in this stage are not abundant, than they are from the other granules appearing in the cell.

While the above mentioned processes have been taking place in the connecting fibers, the radiating fibers and the cytoplasm have undergone important changes. In the larch the radiating fibers apparently grow longer, so that by the time the chromosomes have reached the poles a great many of them may be traced to the plasma membrane. Any evidence of such a fusion of the fibers and the plasma membrane as Nemec described in the onion I was unable to find. In the equatorial plate stage the fibers certainly did not reach to the plasma membrane. Whether the difference in appearance of the radiating fibers is really due to the growth of the existing fibers or to the formation of new ones, I could not determine. In the later stages, shorter fibers may be seen, which are possibly the radiating fibers of the earlier stages, while the longer fibers are newly formed. The increase in number of fibers which such an explanation would demand was, however, not always evident in my preparations. In connection with changes in the radiating fibers there is a disappearance of the kinoplasmic layer heretofore described as lying between the spindle and the cell periphery. It may be, as previously suggested, that the substance is used in the growth of the radiating fibers, though I have no positive evidence that such a relation exists. The fine granular zone also disappears during the metaphases. There is now a tendency for the cytoplasm around the poles of the spindle to assume a finely granular appearance. The significance of such an appearance I have not attempted to explain.

In the onion the relation of the trophoplasm to the spindle remains practically the same as it was in the equatorial plate stage. When the diaster is formed there is a small number of connecting fibers, and the few radiating fibers that existed during the previous stage have not appreciably changed in appearance. During the later stages of the diaster, however, there is an apparent growth of new radiating fibers extending in all directions from the daughter nuclei. This phenomenon I shall discuss in a subsequent paragraph.

Following the formation of the diaster there sets in a series of activities which are concerned immediately with the formation of the cell plate. In the larch, the spindle soon appears to be differentiated into three zones (*fig. 2*), as Went first pointed out in other forms. But I cannot confirm the statement that the darker subnuclear zones take their characteristic appearance from a stainable ground substance between the spindle fibers. A careful study of the preparation from which *fig. 2* was taken has convinced me that the darker portions are due to the structure and arrangement of the spindle fibers themselves. Some of the fibers are undoubtedly thicker in this region than in the equator (*fig. 27, a*). With this differentiation in structure is combined the fact that the bundles have here begun to separate into single fibers (*fig. 2*). The two processes taken together account for the extra density observed. The above facts seem to me to indicate that the kinoplasmic activity preparatory to the formation of the cell plate begins in the region of the nuclei. The thickened appearance of the fibers soon extends throughout their length (*figs. 3* and *27, b*). Concurrent with such thickening the separation of the bundles into single fibers continues until the central spindle has the same appearance throughout. By comparing *figs. 2* and *3* it can be seen that nearly all of the connecting fibers have apparently shortened slightly, leaving clear spaces just under the two daughter nuclei. In many cells there were often observed single connecting fibers in which the above described changes did not seem to be taking place, but they were never numerous in any one cell. Their distribution was not regular. They were as often seen in the central as in the more peripheral parts of the spindle. I shall have occasion to refer to them in the description of later stages. Granules of trophoplasm may often be detected among the ends of the connecting fibers at this stage. These have probably flowed in as the fibers contracted. The process of separation of the fibers described above gives the appearance of an increase in the number of spindle fibers. Whether such an increase actually occurs is doubtful. In the larch the evidence, so far as I have observed it, seems to show that the apparent increase is due entirely to the above mentioned processes. In this connection the hypothesis lately suggested by Strasburger, that the spindle fibers are increased in numbers by splitting, is of interest. His evidence for the hypothesis may be stated as follows: (1) the apparent rapid increase in the number of fibers; (2) the fibers produce a rapid outgrowth of the cell plate; (3) they are often found lying closely side by side. The first and third points seem to me to be readily explained by the facts that I have already described for the larch, viz., the rearrangement of the fibers by the bundles separating into single fibers, and the shortening and thickening of the fibers, resulting in a spindle of denser appearance, with apparently more numerous fibers. Strasburger himself points out that the spindle fibers seem to separate from the daughter nuclei and to become thicker and more densely stained prior to the formation of a cell plate. It should be noted, however, that Strasburger had previously accepted Guignard's doctrine that the connecting fibers are secondary structures formed by the fusion of smaller primary fibers. The splitting of the fibers, in Strasburger's sense, would be essentially the same as the separation of fibers, as described above, with this one exception: in the former case the process is unlimited, for new fibers may continuously arise by the splitting of original fibers, but in the latter the process is limited by the number of fibers making up the bundles. If the splitting hypothesis were true, it would explain, as Strasburger suggested, the appearance of new peripheral connecting fibers, and thereby the growth of the cell plate; but below I shall describe phenomena which seem to indicate that the appearance of new peripheral fibers depends not upon such a multiplication of the original connecting fibers, but upon changes in some of the radiating fibers.

Concurrent with the above described changes in the connecting fibers, the distal portions of the radiating fibers that lie nearest the central spindle and cross at the equator seem to bend in such a way as to come nearer together and to give the appearance of bowed out connecting fibers; but that they are the previously described radiating fibers is evident from a careful study of the preparations. In *fig. 26* I have drawn accurately two such fibers extending one from each nucleus. Here the relation is such as to indicate that the two fibers have fused laterally throughout a part of their length. Whether such a fusion is real or only apparent, I could not determine A careful study of the preparation from which the photograph for *fig. 2* was made shows all stages in the arrangement of radiating fibers, from that shown in *fig. 26* to that in which the fibers extend out into the cytoplasm and cross at the equator in such a way as to form sharp angles (*fig. 24*). The significance of these facts I shall discuss in a later connection. The radiating fibers that do not cross at the equator, so far as I could determine, have suffered no appreciable change. It is important to keep in mind that, with the exception of the presence of the trophoplasmic granules, the changes in the appearance of the spindle which have so far taken place in the larch are due mainly to changes in the existing spindle fibers themselves, and not to the addition of new fibers or of other material.

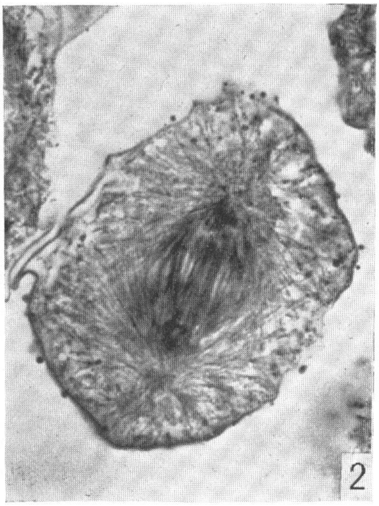
If we turn now to the onion, we note that there is an apparent increase in the number of connecting fibers (*figs. 12, 13, 14*). *Fig. 13* represents the same stage in the onion as that represented by *fig. 2* in the larch. The slight differentiation of the spindle into zones may be seen. While the smaller number of spindle fibers renders such a differentiation less conspicuous, it is evident that it is caused in the same fashion as in the larch. A fact of importance to note here is that there are now visible more radiating fibers than could be seen in earlier stages. The photograph has not brought these out very clearly, but they may be seen by close inspection. These fibers radiate in all directions from the nuclei, but those are more abundant which extend toward the equator. In this connection it may be worth while to point out that these new fibers do not bear exactly the same relation to the spindle as the radiating fibers that exist in the equatorial plate stage. Those were centered not on the nuclei or chromosomes but upon the poles of the spindle. It is possible, however, that they were originally centered upon the mother nucleus, as previously suggested. From a careful comparison of this with earlier stages, I am convinced that there has been a growth of new fibers. It is not impossible that some of these extend into the central spindle, thus increasing the appearance of zonal differentiation, but I could not establish this by actual observation. In an earlier paper Guignard figured such a relation but did not describe it. It seems to be not improbable that such a condition may exist, and that such fibers may grow in length and form new connecting fibers, either by fibers from the opposite nuclei fusing, or by a continuous growth from one nucleus to the other. This process would account for the apparent increase in number of connecting fibers. Still the evidence is too meager to lead to any definite conclusion. There is no convincing evidence that there is any real increase in the number of connecting fibers. Its appearance may be due entirely to the changes which take place in the original fibers, as is the case in the larch. On the other hand, the relatively small number of the connecting fibers in earlier stages seems to show that some new ones have been formed. The question needs further investigation. If there are new connecting fibers formed they seem to act simultaneously with the original fibers in the process of forming the cell plate. The point that seems of most importance to me here is the previously described relation of the radiating fibers to the daughter nuclei, *i. e.*, that they center not upon the poles of the spindle but upon the nuclei themselves. This relation, combined with the fact that the fibers are new formations, may indicate that the nuclei are the metabolic centers for the formation of spindle fibers. Such an hypothesis is further strengthened by the previously described changes in the connecting fibers in the larch, where the increased thickness is first evident near the ends of the fibers, *i. e.*, in those portions nearest the nuclei. The fact that the appearance of the new fibers comes prior to the reconstruction of the daughter nuclei does not invalidate the above hypothesis, for, as Juel has shown in the formation of abortive pollen grains in *Hemerocallis fulva*, single chromosomes may have spindles formed between them and the normal nucleus. Here would seem to be a case in which a chromosome as such acts as a center for spindle formation. While the recent contributions on the subject of spindle formation in higher plants vary greatly in detail they all agree in one particular, viz., that the first indications of a spindle are to be found in connection with the nucleus and that the subsequent prophases take place while the fibers remain in this connection. These facts may be explained by the hypothesis that the nucleus is the center for the formation and activity of the kinoplasm.

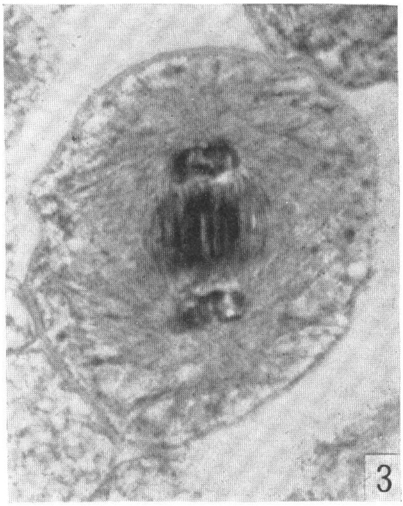
The further history of the equatorial zone in the onion presents a striking difference from its history in the larch. Whereas in the latter it disappears with the rearrangement and change in the connecting fibers prior to the formation of the cell plate, in the former it soon becomes filled with a substance that stains strongly with the orange of the triple stain. The spindle fibers retain their violet color in the same regions, showing that the orange stain is taken by a substance foreign to the fibers (*figs. 14, 15, 28*). In appearance this substance is entirely homogeneous. I could detect no appearance of granules or any other definite structural elements in it. It would seem to be a substance in solution in the living cell. It is a significant fact that the cell walls showed the orange stain in those preparations in which this orange stained substance is most evident in the spindle. In many preparations in which there was a slight excess of violet, the cell walls were stained blue and the substance in the equatorial zone could scarcely be distinguished from the surrounding blue stained protoplasm. By the use of other stains it was often difficult to color or differentiate this substance. In one preparation, stained with eosin and methyl green, in which the methyl green was in excess, all parts of the cell were stained green except the equatorial zone and the cell walls, both of which showed a slight tinge of eosin. In all of the stains used when both the cell wall and the interfilar substance were stained, their color was the same. There were some cases, however, in which the walls were stained while the equatorial zone remained colorless, *e. g.*, slides stained in ruthenium red or iron haematoxylin.

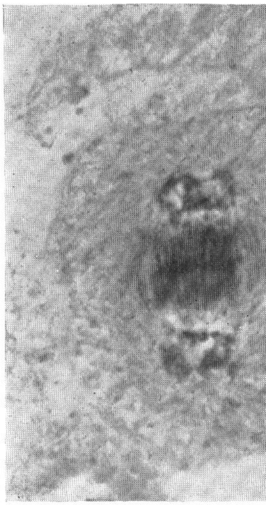
The similarity in staining of this substance to that of the cell walls, together with its presence in the region of the spindle in which the cell wall appears later, I have taken to signify the presence of a carbohydrate substance destined for the formation of the new cell wall. Whether it is at all analogous to the previously described layer around the spindle in the cytoplasm of the larch, I am not able to say. The fact may be of some significance in this connection, that in a late stage of division in the larch a similar orange layer appears in the cytoplasm around each daughter nucleus. Possibly it shows the presence of material destined for the formation of the walls of the pollen grains. This fact may be correlated with the fact that the division of the pollen mother cell often does not follow the first nuclear division. But in the stages showing the young permanent cell plate, such a substance was generally invisible, though in some cells a slight indication of it was seen. There would seem to be in these cells very slight or no aggregation of reserve cell-wall material. It is interesting to note that in the dividing pollen grains of Iris and Hemerocallis no orange stained interfilar substance was seen. Here, of course, there is no cell wall formed between the two cells.

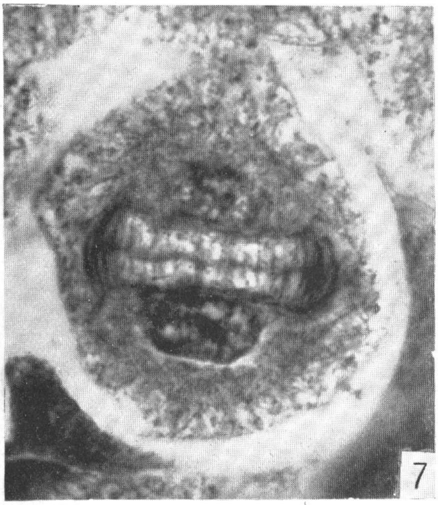
The relation of the spindle fibers to this orange substance is worthy of notice. As above mentioned, they retain their characteristic color, but they often appear greatly attenuated in this region. It would seem that the substance had crowded them into such a condition (*figs. 28, 29*), and that it retarded the previously described processes of thickening and separating of the fibers.

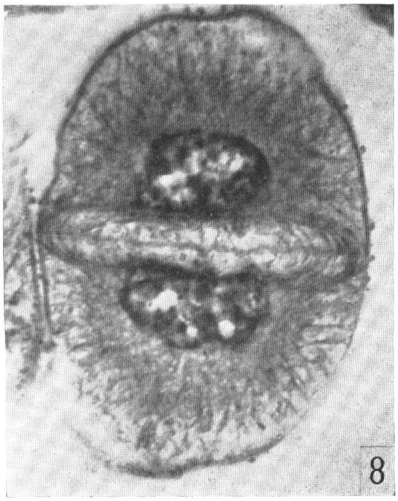
The staining qualities of the interfilar substance agree with what Farmer and Williams have described for the protoplasm of the neutral zones between the daughter nuclei of the future oospheres of Fucus. In the Saprolegniaceæ Trow has suggested that the so-called cellulin granules are a form of reserve cellulose, which may be used to close the opening made by the breaking of a hypha. I have seen preparations of Saprolegnia in which the young cell wall cutting off the sporange stains strongly with

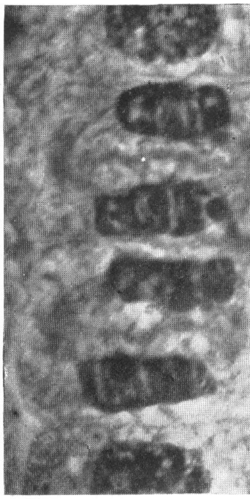

2

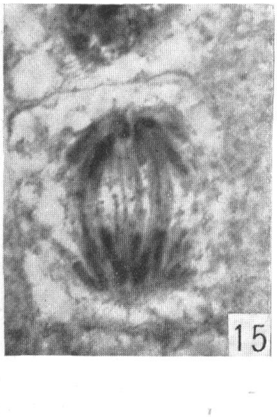

3

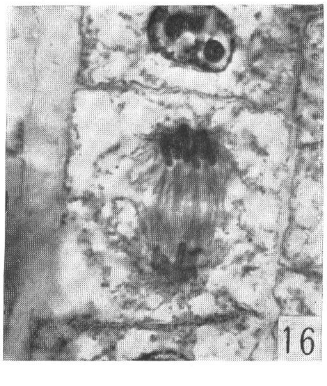


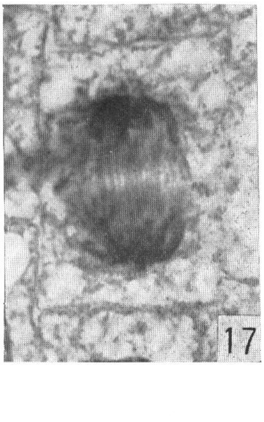

7

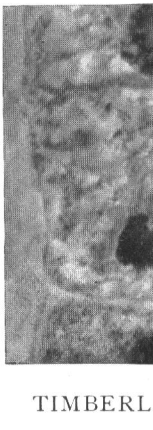

8



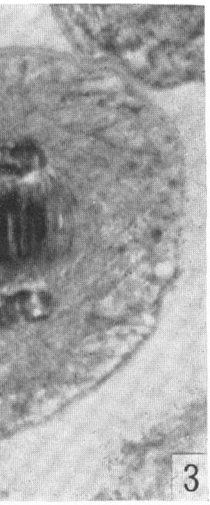

15

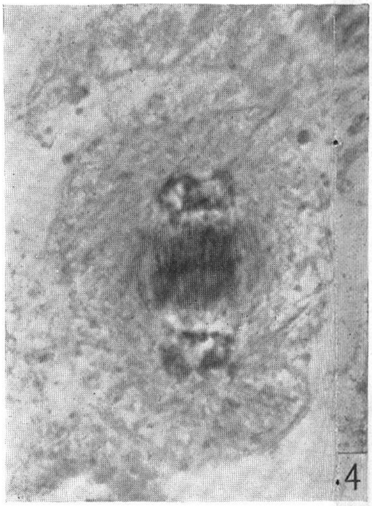

16

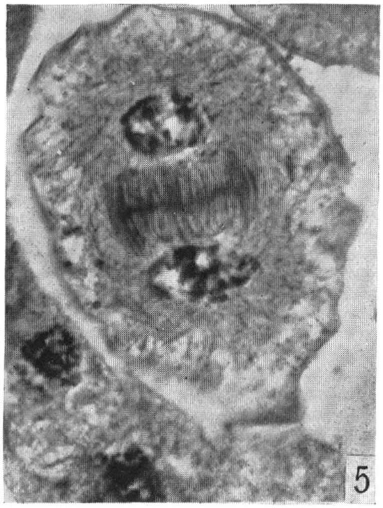

17

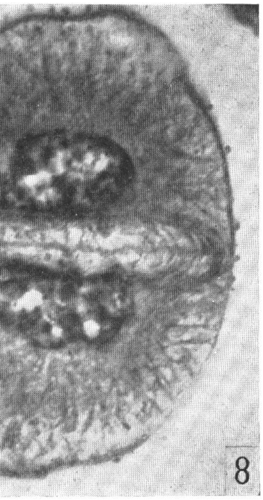


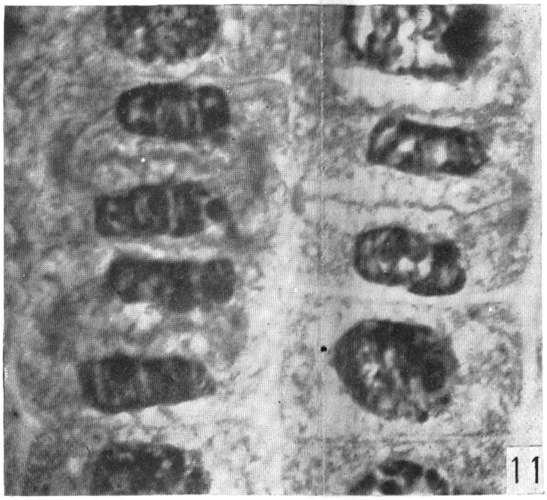
TIMBERL

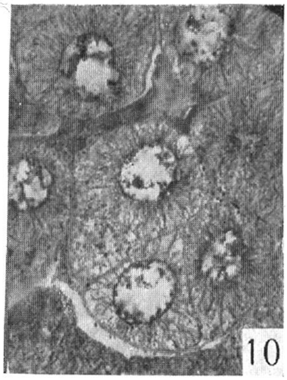

3

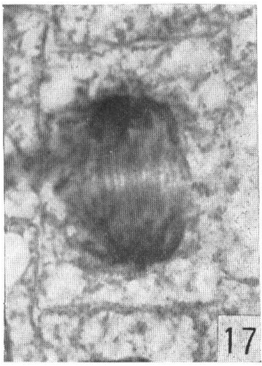

4

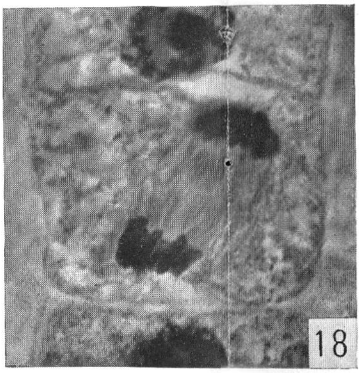

5

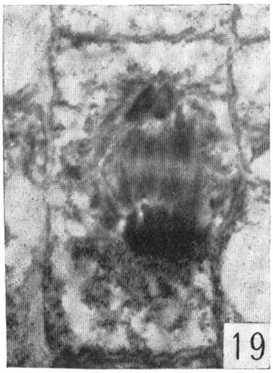

8


11


10

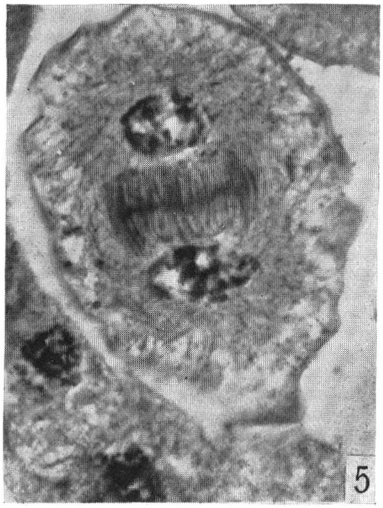

17

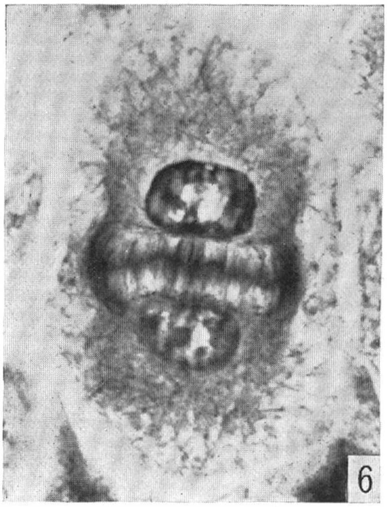

18

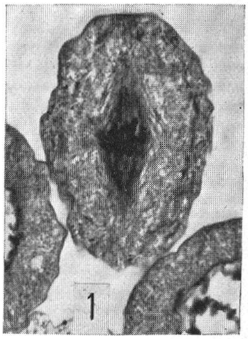

19

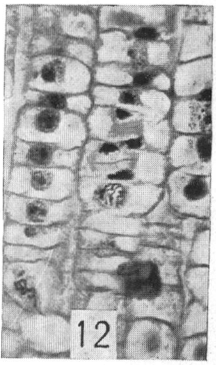
TIMBERLAKE on CELL PLATE

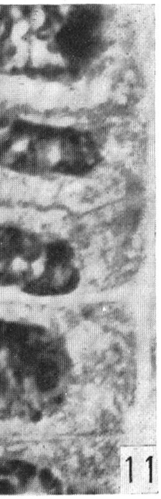
PLATE VIII

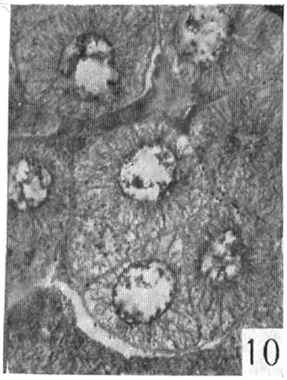

5

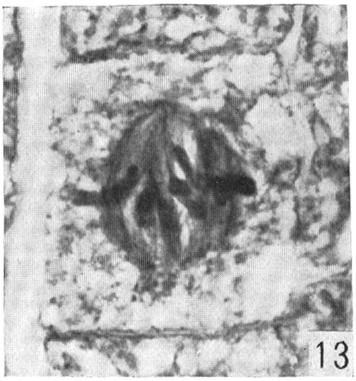

6

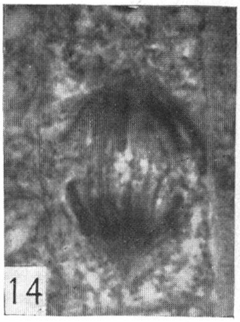

1


12

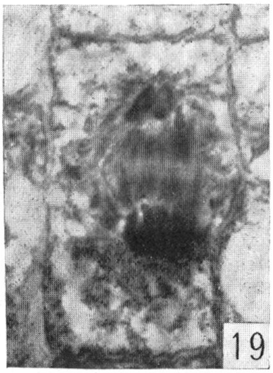

11

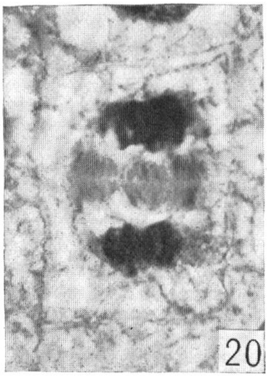

10

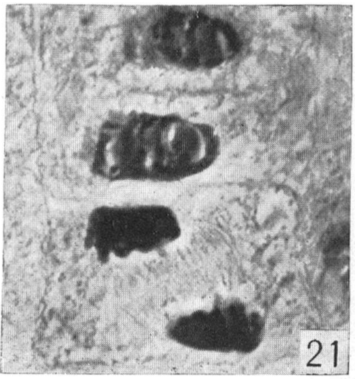

13


14

18


19


20


21

LL PLATE

orange, and strongly orange stained granules are often apparently imbedded in the adjacent thick plasma membranes. The above facts seem to indicate that there may be in the protoplasm some form of reserve carbohydrate in readiness for the formation of a cell wall. I shall hereafter use the term carbohydrate material in speaking of the interfilar substance. The further history of this material is connected with the subsequent stages in the development of the cell plate. The essential difference thus far between the larch and the onion is that in the former the processes preparatory for cell division have been mainly carried on in the already existing fibers, while in the latter there has been a formation of new fibers and an aggregation of carbohydrate material in the equatorial region of the central spindle.

University of Wisconsin.

(*To be concluded.*)